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MORPHOLOGICAL AND MOLECULAR EVIDENCE CONCERNING THE RELATIONSHIP OF *LUPINUS POLYPHYLLUS* AND *L. WYETHII* (FABACEAE)

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Abstract

Lupinus polyphyllus and *L. wyethii* are closely related members of a species complex widely distributed in western North America. Principal components analysis of morphological characters showed that these two taxa intergrade extensively, with many intermediate forms occurring. DNA sequences of the internal transcribed spacer (ITS) region in 28 individuals were variable at five base positions, both within and between taxa; some of these sequences have also been reported from related species. Molecular variation showed a geographic pattern but did not strongly reflect morphological differences. Morphological features that have been used to separate species in this group may primarily reflect local adaptation rather than underlying phylogenetic divergence. The lack of clear differentiation between these two lupines suggests that they are best treated as varieties of a single species, *L. polyphyllus*.

Key Words: *Lupinus polyphyllus, Lupinus wyethii,* principal components analysis, ITS, intraspecific variation.

The genus *Lupinus* (Fabaceae) comprises at least 200 species, occurring mainly in the New World (Dunn and Gillett 1966; Käss and Wink 1997a; Ainouche and Bayer 1999). *Lupinus* is especially diverse in western North America, where it includes several taxonomically difficult species complexes (Dunn and Gillett 1966; Barneby 1989; Ainouche and Bayer 1999). Members of these difficult groups exhibit plasticity in taxonomically important characters, and often appear to be separated by weak reproductive barriers, leading to abundant individuals of intermediate morphology (Dunn and Gillett 1966). Polyploidy (reported for a few species; Phillips 1957) and hybridization may also contribute to this taxonomic complexity.

One such group is centered on *Lupinus polyphyllus* Lindl. (large-leaved lupine), a widespread polymorphic species found in moist meadows throughout western North America. The taxa of the *L. polyphyllus* group intergrade extensively. Some authors (Barneby 1989; Hickman 1993) treat the *Lupinus polyphyllus* complex as a single, highly variable species; others (Dunn and Gillett 1966; Hitchcock and Cronquist 1973; Douglas et al. 1999) recognize one or more distinct species in addition to *L. polyphyllus*. Species that have been segregated from *L. polyphyllus* by various authors include *L. ammophilus* Greene, *L. burkei* S. Wats., *L. holmgrenanus* C. P. Smith, *L. prunophilus* M. E. Jones, *L. saxosus* Howell, *L. subsericeus* Robinson ex Piper, and *L. wyethii* S. Wats. One of the most detailed recent treatments of this complex is provided by Barneby (1989), who recognizes six varieties of *L. polyphyllus,* separated largely on size characters and habitat differences: var. *ammophilus* (Greene) Barneby, var. *burkei* (Wats.) Hitchcock, var. *humicola* (A. Nels.) Barneby, var. *prunophilus* (Jones) Phillips, var. *saxosus* (Howell) Barneby, and var. *polyphyllus*. It is clear from Barneby's discussion that in addition to within-group variation, the taxa of the *L. polyphyllus* complex are connected through intermediate forms with species of several related groups, including *L. argenteus, L. nootkatensis, L. latifolius* and *L. arcticus* (Dunn and Gillett 1966; Barneby 1989).

One of the most strongly marked taxa of the *L. polyphyllus* group is *L. wyethii* Wats. (Wyeth's lupine), which occurs in steppe and montane habitats from southern British Columbia to Oregon, Montana, Wyoming and northern Nevada. *Lupinus wyethii* is known from fewer than five localities in B.C. and is considered critically imperiled, with a provincial ranking of S1 (Douglas et al. 2002). It has been variously treated as a distinct species (Douglas et al. 1999; Hitchcock and Cronquist 1973) or as the variety *L. polyphyllus* var. *humicola* (A. Nels.) Barneby (Barneby 1989). Because conservation priorities lie in protecting species that are genetically and taxonomically distinct, an understanding of the taxonomic status of *L. wyethii* is important for determining the priority it receives in conservation planning (Edward 1997).

Lupinus polyphyllus and related taxa have been included in recent molecular phylogenetic studies of *Lupinus* based on ITS and other DNA regions (Käss and Wink 1997a; Ainouche and Bayer 1999; Ree et al. 2004) and in each of these studies, *L. polyphyllus* is placed along with most other western North American lupines in a monophyletic New World clade. However, all three studies reported

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TABLE 1. MORPHOLOGICAL CHARACTERS OF *LUPINUS POLYPHYLLUS* AND *L. WYETHII* USED IN PRINCIPAL COMPONENTS ANALYSES. Means, standard deviations and ranges of variation are given for sampled specimens of each taxon.

	Lupinus polyphyllus		*Lupinus wyethii*	
Characters and units of measurement	Mean ± SD	Range	Mean ± SD	Range
Plant habit				
Plant height (cm)	55.60 ± 18.83	21–101.5	34.62 ± 8.06	25.5–56
Stem thickness 5 cm below inflorescence (mm)	0.32 ± 0.10	0.2–0.6	0.24 ± 0.06	0.1–0.3
Number of lateral branches	1.17 ± 1.31	0–5	1.83 ± 2.50	0–10
Pubescence				
Length of stem trichomes (mm)	0.75 ± 0.33	0.3–1.5	0.89 ± 0.28	0.4–1.8
Trichome density on stem (0 = none to 5 = very dense)	2.51 ± 0.88	1–4	3.26 ± 0.54	2–4
Trichome density on leaf upper surfaces (0 to 5)	0.49 ± 1.00	0–3.5	3.07 ± 0.79	1–4
Trichome density on leaf lower surfaces (0 to 5)	2.59 ± 0.60	1.5–4	3.82 ± 0.53	2.5–4.5
Trichome density on keel (0 to 5)	0.60 ± 1.08	0–3	2.91 ± 0.90	0–4
Leaves				
Number of basal leaves	2.55 ± 1.78	1–9	10.52 ± 11.70	1–40
Number of cauline leaves				
Petiole length (cm)	8.36 ± 5.16	2–27	10.57 ± 8.19	2–37
Number of leaflets	15.67 ± 7.76	4–40.2	9.89 ± 2.99	4.6–18.5
Length of middle leaflet (cm)	10.2 ± 2.7	6–15	9.0 ± 1.1	7–11
Length/width ratio of middle leaflet	5.87 ± 1.78	2.5–10.8	3.75 ± 1.41	2.1–7.6
Shape of leaflet tip (1 = acute,	5.73 ± 1.76	3–10.8	7.61 ± 2.52	2.7–14.3
2 = rounded to mucronate, 3 = obtuse)	2.40 ± 0.79	1–3	2.98 ± 0.10	2.5–3
Inflorescence				
Number of racemes	1.17 ± 0.56	1–4	2.17 ± 1.67	1–7
Number of flowers in terminal raceme	54.07 ± 26.93	12–118	31.29 ± 9.29	15–52
Length of terminal raceme (cm)	16.04 ± 6.94	3.8–34.3	9.73 ± 4.01	4.2–19.8
Pedicel length (mm)	0.66 ± 0.23	0.2–1.2	0.57 ± 0.15	0.3–0.9
Flowers (measurement from lowermost open flower)				
Banner length (mm)	1.08 ± 0.16	0.7–1.4	1.02 ± 0.15	0.7–1.3
Keel length (mm)	1.46 ± 0.22	0.8–2	1.35 ± 0.17	1.0–1.6
Ratio of upper and lower calyx lobe lengths	0.85 ± 0.13	0.57–1.13	0.88 ± 0.10	0.69–1

largely unresolved relationships within this group. Ree et al. (2004), in phylogenetic analyses based on ITS and two paralogous LEGCYC genes, found that two accessions of *L. polyphyllus* were interspersed with the related species *L. andersonii, L. sericeus* and *L. argenteus.* The findings of these authors suggest that the western lupines are recently diverged in comparison with other lupine groups, and may also imply ongoing hybridization and reticulate evolution.

In this paper, we present morphological and molecular evidence concerning the taxonomic relationship of *L. polyphyllus* and *L. wyethii.* We used (1) multivariate analyses of morphological characters from herbarium specimens to assess the divergence of these taxa and the usefulness of various traits for distinguishing them, and (2) sequence variation in the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene to assess differences between the two taxa, intraspecific variation, and the geographic distribution of genetic variants. We also assessed the relationship of these two taxa to other western North American lupines by comparing our data to the published ITS1 and ITS2 sequences available for this group.

METHODS

Morphological Analyses

For analysis of morphological variation, specimens of both taxa were obtained from the following herbaria: OSC, UBC, UVIC, V, and WTU. We included specimens from British Columbia, Washington, Oregon, Idaho, and Montana. Although most of the accessions of *L. polyphyllus* used in this study were not identified to subspecies or variety, they represented a large range of morphological variation within the species.

We compiled an initial list of 75 morphological characters (see Edward 1997 for complete list), including all those that have been used to distinguish *L. polyphyllus* and *L. wyethii.* From these we selected 22 characters, including the characters used to separate the two taxa, for the final analyses (Table 1). We included characters that (i) represented all parts of the plant, (ii) were obtainable from all specimens, (iii) could be determined repeatably, (iv) did not require destructive sampling, and (v) were not highly correlated with other characters ($r < 0.9$). When multiple measurements of a character were made from a specimen, these were averaged

TABLE 2. *LUPINUS* SPECIMENS USED FOR DNA ANALYSIS.

Species	Location and collector	Herbarium	GenBank #
L. polyphyllus	Alaska, Mt. Marathon, *J.A. Calder 6207*	V	AY948993
L. polyphyllus	British Columbia, Cronin Mt., *G. Mendel 83*	V	AY948994
L. polyphyllus	British Columbia, Copper City, *G. Mendel 85*	V	AY948995
L. polyphyllus	British Columbia, Liard Hot Springs, *T.C. Brayshaw s.n.*	V	AY949017
L. polyphyllus	British Columbia, Cottonwood River, *T.C. Brayshaw s.n.*	V	AY948996
L. polyphyllus	British Columbia, Summit Lake, *R. Long 2-4-71*	V	AY949018
L. polyphyllus	British Columbia, Ashnola Provincial Forest, *T.C. Brayshaw 77-531*	V	AY949010
L. polyphyllus	British Columbia, Mayne Island, *H. Janszen 1094*	V	AY948997
L. polyphyllus	British Columbia, Port Alberni, *W. Van Dieren 353*	V	AY948998
L. polyphyllus	Washington, Olympic National Park, *W. Van Dieren 518*	V	AY949011
L. polyphyllus	British Columbia, Dewdney Island, *R.T. Ogilvie 8471110*	V	AY948999
L. polyphyllus	British Columbia, Shawnigan Lake, *B. Turner 1486*	UVIC	AY949000
L. polyphyllus	British Columbia, Cowichan River, *B. Chapman 853*	UVIC	AY949012
L. polyphyllus	British Columbia, Nanaimo River, *W. Fleming M-4*	UVIC	AY949013
L. polyphyllus	Oregon, Polk Co., *R. Halse 2844*	UVIC	AY949001
L. polyphyllus	British Columbia, Manning Provincial Park, *G. Rushton s.n.*	UVIC	AY949019
L. polyphyllus	British Columbia, Quesnel, *C. Selzler 11*	UVIC	AY949002
L. polyphyllus	Washington, Chelan Co., *M. Denton 3722*	WTU	AY949020
L. polyphyllus	Idaho, Camas Co., *J.H. Christ 53-27*	WTU	AY949005
L. polyphyllus	Washington, Wallowa-Whitman National Forest, *B. Bafus 365*	WTU	AY949006
L. polyphyllus	Washington, Lewis Co., *S. Gage 29*	WTU	AY949003
L. polyphyllus	Washington, Clark Co., *R. Halse 3872*	WTU	AY949004
L. wyethii	British Columbia, Keremeos, *T.C. Brayshaw 77-1011*	V	AY949014
L. wyethii	Alberta, Kananaskis Valley, *R.T. Ogilvie 9862*	V	AY949007
L. wyethii	Washington, Vernita, *S. Mitchell s.n.*	UVIC	AY949008
L. wyethii	British Columbia, Vernon, *S. Mitchell 481*	UVIC	AY949015
L. wyethii	Wyoming, Teton Co., *C.L. Porter 9325*	WTU	AY949009
L. wyethii	Utah, Duchesne Co., *A. Cronquist 11387*	WTU	AY949016

to give a single value of each character for that specimen. We used a total of 70 herbarium specimens (47 of *L. polyphyllus* and 23 of *L. wyethii*) for multivariate analysis, sampling only well-preserved specimens from which all morphological measurements could be obtained.

Principal components analysis (PCA) of the standardized data was carried out using Statistix for Windows (Analytical Software, Tallahassee, FL). We examined correlations of the first and second principal component (PCA-1 and PCA-2) scores with all morphological characters to determine which characters contributed most to the observed patterns. We also examined correlations of PCA scores and individual morphological characters with latitude, longitude and elevation.

Molecular Analyses

Leaf material was taken, with permission, from herbarium collections at UVIC, V and WTU (Table 2). We selected multiple-leaved specimens that could be sampled with minimum loss of morphological information. Total DNA was isolated using a modified CTAB extraction protocol (Doyle and Doyle 1990; Wheeler 2000). Because we carried out the two phases of this study separately and used different specimen selection criteria, the morphological and molecular analyses were based for the most part on different sets of specimens.

The entire ITS region including the 5.8S gene was amplified by PCR using primers 1406F and 307R (Soltis and Kuzoff 1995). Amplification was carried out in 100 μL reactions, including 1μg genomic DNA, 10% 10× amplification buffer, 0.2 mM dNTPs, 0.25 μM of each primer, 2 units Taq DNA polymerase (GibcoBRL, Burlington, Canada) and 5% DMSO. Amplification reactions were performed on an MJ Research PTC-200 DNA Engine Thermal Cycler as follows: 94°C for 3 min, 30 cycles of 94°C for 30 sec, 55°C for 60 sec and 72°C for 60 sec, and final extension at 72°C for 10 min. PCR products were purified with a QIAquick PCR purification kit, and were then sequenced directly. A DNA Sequencing Ready Reaction Kit (ABI) was used for cycle sequencing reactions, with the two amplification primers and the primer ITS4 (White et al. 1990). DNA sequencing was carried out on an ABI Prism 377 DNA automated sequencer.

ITS sequences were aligned using ClustalX v. 1.8 (Thompson et al. 1997). The limits of the ITS region were determined by comparison with published *L. polyphyllus* sequences deposited in GenBank (Ainouche and Bayer 1999; Käss and Wink 1997a). GenBank accession numbers for sequences reported here are given in Table 2. We compared our results with all published ITS1 and ITS2 sequences for western North American lupines (excluding the 5.8S gene, which was not available for all accessions) to assess the taxonomic and geographic distribution of sequences in this group.

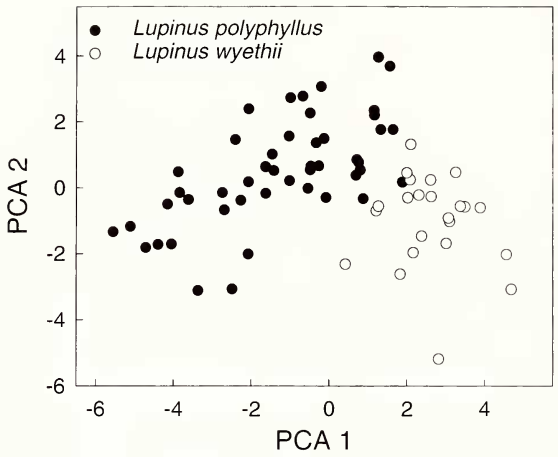

FIG. 1. Principal components of morphological variation in *Lupinus polyphyllus* and *L. wyethii*. Analysis is based on 22 characters and 70 specimens.

RESULTS

Morphological Variation

Principal components analysis (Fig. 1) indicated that *L. polyphyllus* and *L. wyethii* differ morphologically. However, the two taxa clearly form a complete morphological continuum, with many intermediate specimens. Correlations of various characters with principal component scores, particularly along the first axis, reflected the morphological differences between the two taxa. Eleven characters were significantly correlated ($P < 0.05$) with one or both axes (Table 3). Axis 1 scores were positively correlated with trichome density of leaf upper surface and keel, number of basal leaves, and number of racemes; and negatively correlated with several size-related traits including height, leaf characters (petiole and leaflet lengths), and inflorescence characters (length, flower number). Axis 2 scores were negatively correlated with leaf number (both basal and cauline), number of lateral branches, and leaf upper surface trichome density.

Morphological variation in *L. polyphyllus* and *L. wyethii* is summarized for all characters in Table 1. The two taxa differed mainly in pubescence and size. *Lupinus wyethii* was usually more densely pubescent, particularly on leaf upper surfaces (a diagnostic character) and on the keel. It was also generally of shorter stature than typical *L. polyphyllus*, with smaller and often more numerous leaves, a greater tendency toward branching, and shorter, fewer-flowered inflorescences. However, all characters showed at least some overlap, and many were highly variable within each taxon. Pubescence of leaf upper surfaces is considered the diagnostic character for separating these two species. *Lupinus polyphyllus* (characterized as glabrous on leaf upper surfaces) was at least somewhat pubescent on basal and/or cauline leaf upper surfaces in 11 out of 47 plants examined; *L. wyethii* (characterized as pubescent on upper leaf surfaces) was pubescent on leaf upper surfaces in all 23 specimens, but sometimes only sparsely so. Keel pubescence, another diagnostically useful character, was present in all but one of the 23 *L. wyethii* specimens, but lacking in only 38 of the 47 *L. polyphyllus* specimens. Overlap was generally much greater in other morphological characters, and within-plant variation in some traits also suggested that these lupines are phenotypically plastic. Virtually all characters measured in this study showed continuous variation.

Morphology also varied with geographic location and elevation. Latitude of the sampled specimens was positively correlated with PCA-2 score ($r = 0.261$), and longitude was negatively correlated with PCA-1 score ($r = -0.561$). Both latitude and longitude were also significantly correlated with many individual morphological characters. These results reflect in part the different distributions of these taxa, *L. polyphyllus* generally occurring further north and west than *L. wyethii* (Fig. 2a). Elevation showed a significant positive correlation with both principal component axes ($r = 0.610$ with PCA-1; $r = 0.245$ with PCA-2) as well as with many morphological characters, indicating that typ-

TABLE 3. PEARSON CORRELATIONS OF MORPHOLOGICAL CHARACTERS WITH FIRST AND SECOND AXIS PCA SCORES. Only characters with significant correlation coefficients ($P < 0.05$) are included.

Morphological characters	Correlation coefficients	
	PCA 1	PCA 2
Plant height	−0.315	
Number of lateral branches		−0.304
Trichome density on upper leaf surfaces	0.388	−0.425
Trichome density on keel	0.481	
Number of basal leaves	0.446	−0.267
Number of cauline leaves		−0.272
Petiole length	−0.336	
Length of middle leaflets	−0.265	
Number of racemes	0.316	
Number of flowers in terminal raceme	−0.379	
Length of terminal raceme	−0.360	

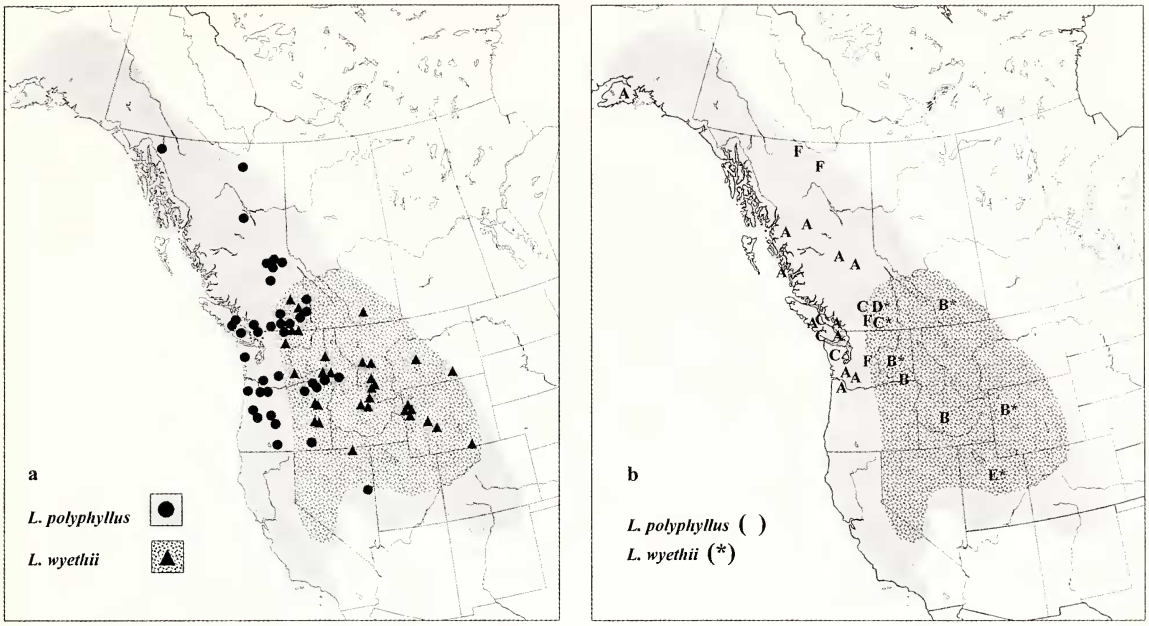

FIG. 2. Ranges of *Lupinus polyphyllus* and *L.. wyethii,* with geographic distributions of morphological and molecular forms. (a) Specimens used for PCA of morphological variation, (b) Sequence variants for ITS found in the two taxa (See Table 4).

ical *L. wyethii* tended to be more commonly found at high elevations

Molecular Variation

The length of the ITS region (including the 5.8S gene) was 628 base pairs in all 28 *Lupinus* specimens examined, with no insertions, deletions or inversions. Sequence chromatograms showed no evidence of mixed sequences within individuals. Five variable base positions were found, each characterized by a single nucleotide polymorphism; three of these occurred in the ITS1 region, and one each in the 5.8S gene and the ITS2 region. This variation yielded six different sequences (variants A to F, Table 4), each differing from the others by a single nucleotide substitution. The most common variant (A) was found in 12 of the 28 plants sampled, and the least common (D and E) were each found in only one individual.

ITS sequences showed some differences between the two taxa (Table 4), but some overlap. Of the six sequence variants found, two (A and F) were exclusive to *L. polyphyllus,* and two (D and E) to *L. wyethii*; the remaining two (B and C) occurred in both taxa. The shared variants were found in 27% of the *L. polyphyllus* specimens and 67% of the *L. wyethii* specimens (Table 4). Within *L. polyphyllus* there was no correspondence between morphological variety and ITS sequence.

The ITS sequence variants showed striking differences in their geographic distribution (Fig. 2b). Variant A, the most common *L. polyphyllus* variant, occurred from coastal Alaska through western B.C. and Washington to northwestern Oregon, well north

TABLE 4. SEQUENCE VARIANTS OF THE INTERNAL TRANSCRIBED SPACER (ITS) REGION IN *L. POLYPHYLLUS* AND *L. WYETHII*, SHOWING BASE POSITION OF NUCLEOTIDE POLYMORPHISMS AND NUMBER OF SPECIMENS IN WHICH EACH VARIANT WAS FOUND.

Sequence variant	Polymorphic base positions in aligned ITS sequence					Number of specimens showing variant	
	116	166	197	373	412	*L. polyphyllus*	*L. wyethii*
A	T	G	G	C	C	12	0
B	**C**	G	G	C	C	2	3
C	T	**T**	G	C	C	4	1
D	T	G	**A**	C	C	0	1
E	T	G	G	**T**	C	0	1
F	T	G	G	C	**T**	4	0

and west of the geographic range of *L. wyethii.* Variant F was also widely distributed, but occurred further east in more continental climates. Variant B, occurring to the south and east, and variant C, of northwestern Washington and southern B.C., occurred in both *L. polyphyllus* and *L. wyethii* where the ranges of the two species overlap. Much of the geographic range that we examined was characterized by particular sequence variants, indicating considerable genetic structure within the two taxa.

Of the six ITS sequence variants we found (Table 4), two (*L. wyethii* sequences D and E) were novel, one (*L. polyphyllus* sequence C) was previously reported from *L. polyphyllus,* and three have been reported from other species (Ainouche and Bayer 1999; Ree et al. 2004). Sequence A from *L. polyphyllus* has also been found in *L. argenteus, L. aridus,* and *L. rivularis.* Sequence B, found in both *L. polyphyllus* and *L. wyethii,* is identical to sequences reported from *L. andersonii, L. argenteus, L. leucophyllus, L. sericeus* and *L. sulphureus.* Sequence F from *L. polyphyllus* is identical to sequences reported from *L. arcticus* and *L. brewerii var. bryoides.* Ree et al. (2004) and Käss and Wink (1997a) found sequence variants for *L. polyphyllus* different from those reported here; altogether, seven ITS variants are known from *L. polyphyllus.*

Discussion

Lupinus polyphyllus and *L. wyethii* are not well differentiated morphologically, as indicated by the range of morphological intermediates found in this study, and the absence of any clear morphological discontinuity separating them (Fig. 1, Table 1). These two taxa have very similar floral features, and are separated only by quantitative differences in size and pubescence of vegetative structures. Such vegetative differences may indicate adaptation of genotypes to local habitats, but may also reflect phenotypic plasticity, a possibility supported by our observations that characters sometimes showed within-plant variation. In either case, caution should be used in giving such differences formal taxonomic recognition.

Lupinus polyphyllus and *L. wyethii* were both variable for ITS, each possessing four of the six sequence variants found. Two variants were present in both taxa. All sequence variants differed by only one base substitution, thus the molecular differences between *L. polyphyllus* and *L. wyethii* were no greater in magnitude than the differences among individuals within *L. polyphyllus* or *L. wyethii.* Comparison of all available ITS sequences for western North American *Lupinus* species (Käss and Wink 1997a; Ainouche and Bayer 1999; Ree et al. 2004) revealed a similar pattern; intraspecific sequence differences exist in all of the other eight species (*L. arboreus, L. arcticus, L. argenteus, L. latifolius, L. lepidus, L. rivularis, L. sericeus,* and *L. succulentus*) for which multiple ITS sequences are available and these sequences were commonly shared by different taxonomic species. The relatively high level of intraspecific variation that we found in *L. polyphyllus* may therefore occur throughout this group.

The ITS sequences available for other western North American lupines also indicated a lack of divergence between recognized species within this group. In their phylogenetic study of *Lupinus* based on ITS, Ainouche and Bayer (1999) identified an apparently monophyletic western North American clade of about 30 species (clade E), supported by a single base-pair insertion in ITS1. This clade was poorly differentiated into subclades, and showed the least sequence divergence of all *Lupinus* lineages. Käss and Wink (1997a) reported a similar lack of divergence in ITS sequences of this group, showing identical ITS sequences in *L. polyphyllus* and several other western species including *L. arboreus, L. arcticus, L. nootkatensis* and *L. perennis.* The additional ITS sequences reported here for *L. polyphyllus* are identical to sequences reported by Käss and Wink (1997a), Ainouche and Bayer (1999), and Ree et al. (2004) for other species, including *L. argenteus, L. sulphureus, L. leucophyllus, L. andersonii, L. breweri* var. *bryoides* and *L. sericeus.* ITS sequences are present in multiple copies in an individual genome and undergo concerted evolution, which can present problems for inferring phylogenetic relationships (Alvarez and Wendel 2003). Irrespective of these problems, however, the occurrence of the same ITS variant in different taxonomic species suggests close relationships among these species. Such a pattern could reflect reticulate relationships resulting from hybridization (perhaps involving polyploidy), or simply recent origins and incomplete divergence of taxa.

In this study, we observed distinct geographic distributions of molecular variants, with both east-west and north-south differences. Other authors (Allen et al. 1996; Soltis et al. 1997; Tremblay and Schoen 1999; Golden and Bain 2000; Dobes et al. 2004) have identified similar patterns of intraspecific molecular variation in other western North American species groups, often reflecting the consequences of recolonization after Pleistocene deglaciation. A striking result of our study was that *L. polyphyllus* and *L. wyethii* accessions from the same geographic region often yielded the same ITS sequence (Fig. 2b), suggesting that few genetic barriers separate the two taxa. Although sampling intensity was low, many ITS variants from other species were also geographically localized.

In summary, the amount of molecular divergence among western North American lupines is low in comparison with other lupine clades, and morphological characters commonly used to distinguish species generally do not coincide with patterns of molecular variation. As other authors have also concluded (Ainouche and Bayer 1999; Ree et al. 2004), this suggests that western North American

lupines are a recently originated group. Many of the morphological species currently recognized in this group appear not to be genetically distinct, and may even be polyphyletic. The available data for these species suggest that there is little basis for subdividing western lupines too finely on the basis of morphological differences. Although *L. wyethii* is one of the most recognizable segregates of *L. polyphyllus,* the morphological and molecular evidence together suggest that it is best treated as *L. polyphyllus* var. *humicola.*

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